In Japan, where theileriosis due to *Theileria sergenti* and *Theileria orientalis* constrains livestock production, ILRAD enjoys close links with two institutes—The Tropical Agricultural Research Centre and the National Institute for Animal Health. Several scientists from these institutes have conducted research at ILRAD for periods of up to two years. Japanese scientists have played key roles in ILRAD's development of monoclonal antibody markers and protein analytical techniques used to define antigenic molecules of theilerial parasites. Employing the markers and techniques on return to their home institutes, these scientists have helped to identify antigens with vaccine and diagnostic potential for the disease caused by *T. sergenti.*

A recent comparison was made between Japanese and African *Theileria* parasites using species-specific diagnostic DNA probes. Results of this work have helped to clarify the species complexity of *Theileria* parasites in both regions. Japanese scientists have also helped ILRAD staff to establish the technology needed to express parasite antigens in a baculovirus expression system for experimental vaccine research.

ILRAD's collaborative links with universities in the US and research laboratories in Australia help to integrate research on tick-borne disease problems in South America, the Caribbean, Asia and the Pacific islands.

For more information, contact Dr. Peter Gardiner, Information & Planning officer.

ILRAD Research Updates

Improved technology for labelling DNA probes

ANIMAL DISEASE control agents and researchers in developing countries have long needed improved methods of accurately detecting parasite infections in animals and precisely identifying the infecting organisms. Vector-borne parasitic protozoa endemic in the tropics have traditionally been detected and identified by staining and microscopic examination of specimens from infected host animals and insect vectors, by propagating parasites in tissue culture and experimental animals and by characterizing the organisms using isoenzymes and serological reagents. Each of these methods has drawbacks, making diagnosis and parasite identification problematical and often unreliable.

THESE TRADITIONAL methods may now be complemented, and in many instances replaced, by new methods employing recombinant DNA reagents. Some of the latter also exploit the polymerase chain reaction, to amplify copies of a given DNA fragment, and hybridization of single strands of DNA with their complementary strands. DNA probes, consisting of single strands of parasite DNA fragments, have now been made to identify many important parasitic protozoa. The probes are conventionally labelled with radioisotopes to detect their binding to parasite DNA. By screening field samples of parasites with a panel of DNA probes for different species and subspecies, laboratory workers are identifying parasites with unequalled precision.

IN MOST THIRD WORLD laboratories, however, use of radioisotopes is constrained by lack of technical expertise and expensive equipment. The latter includes intensifying screens and special refrigerators for amplifying the radioisotope signal at very cold temperatures (–80 °C) and darkrooms for processing the exposed x-ray films. A search was therefore undertaken at ILRAD to find alternative, safer and more convenient methods for labelling DNA probes. Several commercially available kits were evaluated for their ability to reveal parasites in crude samples prepared from infected mammalian hosts and insect vectors. Of the kits tested, the most valuable proved to be one employing digoxigenin molecules with an antibody visualization system (Boehringer Mannheim Biochemica Company, Germany).

Diagnostic tests using this technology require relatively simple laboratory support: signals from the probe bound to parasite DNA are visualized by a change of colour on filter

paper, thus removing the need for x-ray film and darkrooms. The kit is relatively cheap, safe and easy to use. Digoxigenin-labelled probes are stable enough to be used repeatedly for a year or longer and can be preserved in ethanol and sent by ordinary mail to laboratories. This digoxigenin labelling of DNA will simplify and increase the utility of parasite identification based on recombinant DNA probes.

Identifying lethal components of the *Theileria* disease complex

TO IMPROVE CONTROL of tick-borne diseases, scientists need a clear understanding of how they are transmitted in the field. In the case of East Coast fever, for example, they need to know where, when and to what extent *Theileria* parasites occur in tick, wild animal and cattle populations.

Complicating such epidemiological studies are the existence of several species of *Theileria,* not all of which cause disease. *Theileria taurotragi,* for example, is a benign species that is indistinguishable in structure in the tick vector from *Theileria parva,* a parasite that causes lethal infections in cattle. The problem of distinguishing non-pathogenic *Theileria* parasites from mildly and severely pathogenic organisms has, until recently, been a stumbling block to epidemiological research.

USE OF MOLECULAR BIOLOGY techniques is solving that problem. ILRAD scientists have cloned DNA sequences of *Theileria* parasites for use as 'molecular probes' to identify genomic differences among *Theileria* species. They have now developed secondary probes using synthetic oligonucleotides based on identified parasite DNA sequences. Probes made of repetitive parasite DNA sequences were produced at ILRAD several years ago to distinguish *T. parva* and *T. mutans* from other *Theileria* parasites.

More recently, in collaboration with scientists supported by the British Overseas Development Administration and scientists at the Kenya Agricultural Research Institute (KARI), ILRAD staff developed a panel of probes targetted to parasite ribosomal RNA sequences. Use of these probes is enabling scientists to distinguish *T. parva* species with unequalled precision, including two species economically important in Africa and Asia, *T. parva* and *T. annulata,* respectively, *T. mutans,* which is mildly pathogenic, and the benign *T. taurotragi.*

USING THE NEW PROBES, scientists from ILRAD and Japan's Tropical Agricultural Research Centre (Tsukuba) recently determined that a species of *Theileria* isolated by KARI scientists and causing disease in cattle in Kenya (Naivasha) is *Theileria buffeli,* a parasite formerly known to occur only in Asia, where it also causes disease. It is not yet known what species of tick transmits this parasite in Africa, but the discovery of its occurrence on the continent will help researchers to establish the dynamics of tick-borne diseases and to predicate more accurately where the diseases may spread.

Role of macrophages in development of anaemia in trypanosomiasis (Ph.D.Thesis)

The engulfing of erythrocytes (red blood cells) by macrophages is known to be one of the primary causes of anaemia in animal trypanosomiasis. To analyse the mechanisms of the phagocytosis of erythrocytes, an *in vitro* assay was established in which peripheral blood monocytes were purified and incubated with ^{51}Cr-labelled erythrocytes. The release of ^{51}Cr was used as a measure of erythrophagocytosis.

Macrophages from trypanosome-infected cattle had a greater rate of erythrocyte destruction than those from uninfected cattle. Erythrocytes from infected cattle were more prone to destruction by macrophages from both trypanosome-infected and uninfected cattle. Erythrophagocytosis by peripheral blood-derived macrophages, bone marrow